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COMPARISONS AND CONTRASTS IN HOST-FORAGING STRATEGIES OF TWO LARVAL PARASITOIDS WITH DIFFERENT DEGREES OF HOST SPECIFICITY

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Abstract—In theory, the degree of specificity of the signals a parasitoid species needs to successfully locate its host correlates with its level of specialization. We examined this question by comparing the foraging strategies of two parasitoids that differ in their host ranges. In wind-tunnel experiments, we investigated how systemically released herbivore-induced volatiles were used by the generalist parasitoid, *Cotesia marginiventris* (Cresson) and the specialist, *Microplitis croceipes* (Cresson). We determined the relative influence of these volatiles as compared to other signals emitted in the host orientation of the two parasitoids. Both the generalist and the specialist parasitoid strongly preferred *Spodoptera exigua* (Hübner) leaf-induced systemic plants over undamaged plants when no other information was available. When wasps were given a choice between leaf-induced and undamaged plants carrying other plant- or host-related materials, the responses differed for the two species. *C. marginiventris* appeared to cue primarily on recent damage volatiles, whereas *M. croceipes* appeared to cue primarily on host frass volatiles. However, recent damage on previously leaf-induced plants, was strongly preferred to recent damage on plants previously damaged by both species. When plants were induced at the squares by *Helicoverpa zea* (Boddie), only *M. croceipes* exhibited a preference for these plants over undamaged plants. The adaptive

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significance of the behaviors as related to dietary specializations of the parasitoids is discussed.

Key Words—Hymenoptera, Braconidae, *Microplitis croceipes, Cotesia marginiventris, Gossypium hirsutum*, volatile chemicals, systemic, generalist, specialist, host location, wind tunnel.

INTRODUCTION

Parasitoids exhibit multiple foraging strategies that are shaped by the host/plant system with which they interact. One current view of the evolution of foraging strategies in parasitoids is that the degree of specialization at a particular trophic level sets the degree of specificity of the information needed for successful foraging (Vet and Dicke, 1992). The greater the number of hosts or plants a parasitoid is able to use, the less specific the information it needs and vice versa. Because specialists depend upon only a few host species, they are expected to benefit more from a highly efficient host detection system than generalists whose wide host range makes detection of a particular host species less crucial. When compared with generalists, specialist parasitoids are expected to use more specific signals, as they need information on host identity, presence, and availability. A few studies have addressed this aspect of host/parasitoid interactions. Vet et al. (1993) compared the response of the specialist *Leptopilina boulardi* and the generalist *Leptopilina heterotoma* to larval extracts from six different drosophilid larvae. They found that the behavioral response to the kairomone reflected the dietary breadth of the two parasitoids, as the specialist displayed a more specific response than the generalist. Geervliet et al. (1996), however, failed to demonstrate such a relationship when they compared the innate long-range host discriminative abilities of the specialist *Cotesia rubecula* and the generalist *Cotesia glomerata.* Neither could discriminate volatiles from plants infested with host and nonhost species. In a comparative study between the generalist parasitoid, *Campoletis sonorensis*, and the specialist, *Microplitis croceipes*, Elzen et al. (1987) found that the specialist parasitoid was attracted by volatiles from feces of its host, *Heliothis virescens*, whereas the generalist was not. These varying results could be the consequence of differences in the bioassays used but also in the volatiles tested.

For each plant–herbivore complex, volatile signals used by foraging parasitoids can originate from the plant, the host, or from an interaction between the two. In a few plants (Dicke et al., 1990a; Turlings et al., 1990; Dicke, 1994; Loughrin et al., 1994; McCall et al., 1994; Takabayashi et al., 1991, 1994) the latter comprises passive release volatiles (i.e., constitutive volatiles emitted upon mechanical damage) as well as induced volatiles (i.e., volatiles emitted as a delayed response to herbivore feeding damage only). Studies with lima beans (Dicke et al., 1990b, 1993), corn (Turlings and Tumlinson, 1992), and recently cotton (Turlings et al., 1995; Röse et al., 1996) have shown that induced volatiles were released not only locally by the damaged leaf, but also systemically in undamaged parts of the plant. Chemical analysis with corn (Turlings et al., 1993a) indicated that the composition of the systemically released herbivore-induced volatiles did not differ significantly with different species of herbivores. Moreover, behavioral experiments with the parasitoids *Cotesia marginiventris* and *M. croceipes* showed that neither species was able to discriminate between volatiles systemically emitted by caterpillar- (*Spodoptera exigua*) or grasshopper- (*Schistocerca americana*) induced plants. Similarly, in cotton, chemical analysis of volatiles systematically released by plants damaged by different species of caterpillars revealed no significant differences, (Röse, unpublished data). Thus, herbivore-induced signals systematically emitted by corn or cotton plants do not appear to carry any specific information on the identity of the herbivore causing the damage. Because of this lack of specificity, systemically released herbivore-induced volatiles do not reliably indicate host presence for specialist parasitoids and therefore could be used differently by generalist and specialist species.

In the present study, we investigated how such volatiles are used in the foraging strategies of two related solitary endoparasitoids that differ in dietary specializations: *M. croceipes* (Hymenoptera: Braconidae), a specialist that parasitizes a limited number of related host species (*H. virescens, Heliothis subflexa, Helicoverpa zea*), and *C. marginiventris* (Hymenoptera: Braconidae), a generalist with a wide range of lepidopterous hosts, including *S. exigua, S. frugiperda* and *Heliothis/Helicoverpa* spp. We questioned the role of systemically released volatiles in the long-range orientation of the two parasitoids and their shifting influence as wasps are exposed to other plant- or host-related signals.

We restricted the study to two lepidopterous herbivores, *S. exigua* and *H. zea*. These species are significant pests of cotton but occur also on a wide variety of cultivated and noncultivated plants (Pearson, 1982; Stadelbacher et al., 1986). *M. croceipes* attacks caterpillar hosts on as many as 24 different host plants (Eller, 1990) while *C. marginiventris* attacks hosts on 30 different species (Turlings, 1990). The two herbivore species exhibit different feeding behaviors. The first three instars of *S. exigua* feed in groups, usually on the underside of the leaves (Poe et al., 1973). *H. zea* larvae, conversely, are solitary feeders. On cotton, their preferred plant parts are squares, flowers, and bolls (Wilson and Gutierrez, 1980). The feeding behavior of early *S. exigua* instars evokes a systemic response in cotton plants (Röse et al., 1996). After 48 hr of continuous damage of the lower leaves, the upper undamaged leaves systemically released (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E*)-β-farnesene, (*E*,*E*)-α-farnesene, and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. All these compounds are induced by herbivore damage and are not released in detectable amounts by undamaged plants. Based on these results, we used *B. exigua* larvae to obtain plants systemically releasing herbivore-induced volatiles.

We investigated whether the specialist parasitoid *M. croceipes* is able to discriminate between volatiles systemically released from nonhost induced plants and volatiles released by undamaged plants. We then determined the primary host-finding cues used by a specialist as compared to a generalist parasitoid. Finally, we questioned whether the natural feeding behavior of *H. zea* larvae triggers the release of parasitoid-attracting volatiles from undamaged parts of cotton plants.

METHODS AND MATERIALS

Hosts

S. exigua and *H. zea* eggs were obtained from the rearing facilities at the IBPMRL, USDA-ARS, Tifton, Georgia. Larvae were fed on a laboratory-prepared pinto bean diet and held in a climatic room at 25°C, 14L:10D, and 70% relative humidity until used for experiments.

Parasitoids

M. croceipes were reared on *H. zea* larvae according to the procedure of Lewis and Burton (1970). *C. marginiventris* were reared similarly on *S. frugiperda* larvae. Both species were reared and held at 25°C, 14L:10D and 70% relative humidity. Under these conditions, adult *M. croceipes* and *C. marginiventris* emerged 17–23 days and 15–21 days after parasitization, respectively.

All experiments were conducted with mated *M. croceipes* and *C. marginiventris* females, 2 and 4 days old, respectively. Unless stated otherwise, females were given an oviposition experience with a *H. zea* larva fed artificial diet immediately prior to being bioassayed.

Plants

Cotton plants (*Gossypium hirsutum*, Deltapine 90 variety) were grown in individual pots in a greenhouse at 25°C ± 10, 15L:9D, 60 ± 20% relative humidity. Seeds were planted in a 1:1 mixture of peatmoss (Promix Bx) and potting soil fertilized with Osmocote. Eight- to 10-week-old plants were used in the experiments. Immediately before beginning the experiments, the stem was submerged in water and cut to remove the terminals consisting of the upper four to five leaves. These terminals were placed in a 125-ml water filled Erlenmeyer flask for use in the bioassays.

Wind Tunnel

A 50 × 50 × 120-cm wind tunnel as described by Drost et al. (1986) was used. Experiments were conducted at a wind speed of 40 ± 2 cm/sec and at 25 ± 2°C and 40 ± 10% relative humidity.

Volatile Sources Tested

The following volatiles sources were used in the wind-tunnel dual choice tests described in the next section:

Terminals from Leaf-Damaged Plants (Leaf-Induced Plants). The three lower leaves were damaged with *S. exigua* larvae, 48 hr prior to bioassay (Figure 1). Four third instars were contained in a 6-cm-diameter screened cage placed on each of the three leaves. Since only the tops of the plants were tested, damaged leaves were always absent in the experiments.

Terminals from Undamaged Plants (Undamaged Plants). Plants were never exposed to caterpillar damage (Figure 1). When they were tested against a square-induced plant, three to four squares were removed and the excisions covered with paraffin. Undamaged plants were kept in close proximity to damaged plants during the 48-hr damage period.

Recently Damaged Leaves (Recent Damage). Depending on treatments, recently damaged leaves either originated from undamaged or leaf-induced plants. One leaf was damaged by 10–15 first and second instar *H. zea* for less than

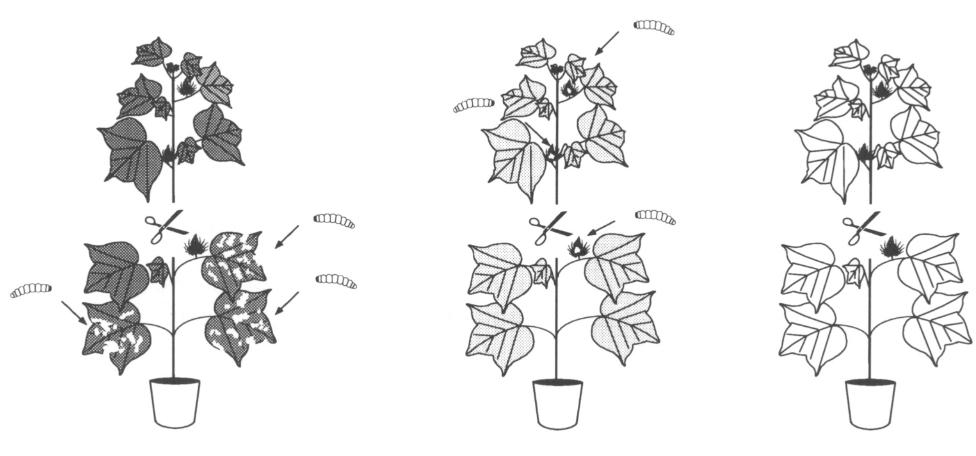

FIG. 1. Schematic representation showing procedure for volatile sources. Cut off terminals of cotton plants were used. (For more details, see "Volatile Sources Tested".)

3 hr. When the leaf was added to another plant, the stem was cut in water and wrapped in moist cotton wool. Host-related products such as feces or silk were removed with a brush.

Frass Obtained from Larvae Feeding on Cotton Plants (Plant Frass). Frass was collected from first and second instar *H. zea* feeding on cotton plants for 24 to 36 hr. Immediately prior to the experiments, the frass was humidified, mashed, and applied with a brush to one leaf of the plant tested. Approximately 3.5 ± 0.5 mg (dry weight) of frass were applied.

Frass Obtained from Larvae Feeding an Artificial Diet (Artificial Frass). Frass was collected from first and second instar *H. zea* feeding on pinto bean diet for 24–36 hr. Artificial diet frass was prepared and applied as described for plant frass.

Terminals from Square Damaged Plants (Square-Induced Plants). Three to four cotton squares were damaged by *H. zea* larvae 48 hr prior to bioassays (Figure 1). One third instar was placed on each square. In order to avoid possible contamination of plants by host-related products, squares were contained in closed 30 ml plastic cups. Damaged squares were excised immediately prior to testing and the excisions covered with paraffin.

Bioassays

Two plant terminals were placed at the upwind end of the wind tunnel. At the beginning of the test, a female, held in a shell vial, was released at the downwind end. The vial was positioned with its opening directed towards the center of the two volatile plumes generated by the terminals. The first plant on which the female landed was recorded. Females were given a maximum of three chances to land on a plant. If they landed anywhere else in the wind tunnel three consecutive times, they were reported as making an incomplete flight. *M. croceipes* and *C. marginiventris* females were tested alternatively. For each combination tested, 10 females of each species were bioassayed and four replications on different days were made. All females were used only once. Plants were switched from one side to the other after *M. croceipes* and *C. marginiventris* each completed five tests.

In order to assess the role of systemically released signals relative to other signals emitted, the following dual-choice tests were performed using volatile sources obtained as previously described:

(a) leaf-induced plant versus undamaged plant; (b) leaf-induced plant versus undamaged plant + recent damage—a recently damaged leaf obtained from a previously undamaged plant was added to the undamaged plant; (c) leaf-induced plant + recent damage versus undamaged plant + recent damage—a recently damaged leaf obtained from a previously undamaged plant was added to the undamaged plant and to the leaf-induced plant; (d) leaf-induced plant with recent damage versus undamaged plant with recent damage—one leaf of the leaf-induced plant and one leaf of the undamaged plant were damaged prior to testing; (e) leaf-induced plant versus undamaged plant + plant frass—Plant frass was applied to one leaf of the undamaged plant; (f) leaf-induced plant versus undamaged plant + artificial frass—artificial frass was applied to one leaf of the undamaged plant. In this treatment, only naive females (never exposed to host or host products prior to testing) were used in order to avoid possible learning and orientation to artificial diet related volatiles in the frass. Treatments a and e were repeated using naive females to permit accurate comparisons of results; and (g) square-induced plant versus undamaged plant.

The following treatments were performed on the same days: treatments a, b, c, and d; treatments a and e; treatments a, e, and f (with naive females); and treatments a and g.

In order to verify if the undamaged part of damaged plants emitted herbivore-induced volatiles and to account for eventual daily variations, experiments were always preceded by a control choice test with a leaf-induced and an undamaged plant (treatment a). Only when the leaf-induced plant was preferred over the undamaged plant (which happened in over 95% of the cases), were subsequent tests conducted using these plants. Data obtained during these control choice tests are included in the figures, and serve as a reference for comparisons among treatments.

Data Analysis

Results of all dual choice tests were analyzed with chi-square tests. The Yates correction for continuity was applied (Zar, 1984).

RESULTS

In all the tests, the overall rate of response of both parasitoids was high. When given three chances to land on a plant, only 1.6% of *M. croceipes* and 8.2% of *C. marginiventris* made incomplete flights in the wind tunnel.

Leaf-Induced Plant Versus Undamaged Plant. Volatiles emitted by *S. exigua* induced plants were attractive to female parasitoids. Both *M. croceipes* and *C. marginiventris* exhibited a strong preference for leaf-induced plants (Figures 2A and 3A). They were chosen by over 80% of females that made complete flights. The responses of both parasitoids to this dual choice test were consistent (compare Figures 2, 3, 4, and 6).

Leaf-Induced Plant Versus Undamaged Plant + Recent Damage. When a recently damaged leaf was added to an undamaged plant, no significant difference was found in *M. croceipes* choice between this combination and the leaf-induced plant (Figure 2B). However, female preference for leaf-induced plants appeared to decrease (compare Figures 2A and B) ($\chi^2 = 27.0$, $P < 0.001$,

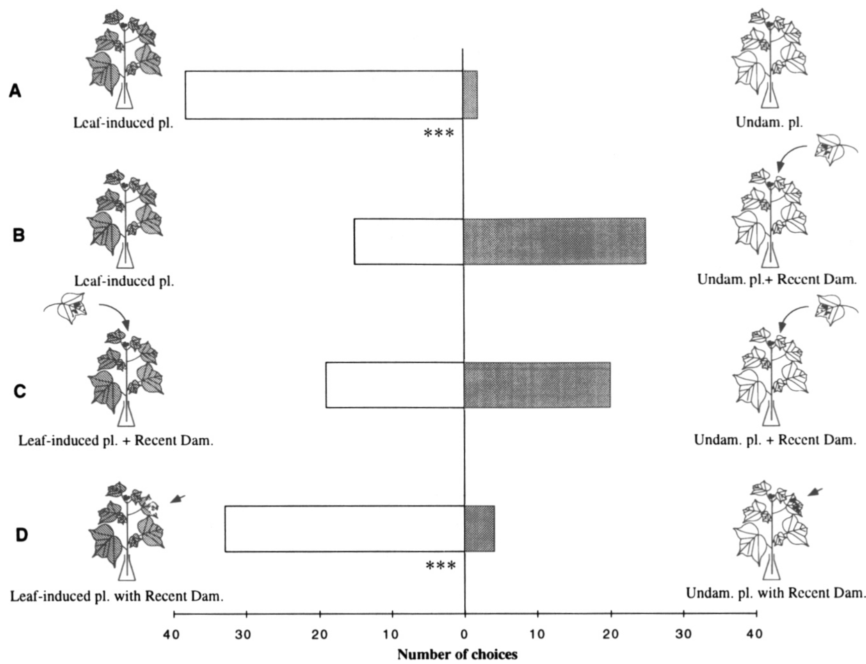

FIG. 2. Flight responses of *M. croceipes* females in wind-tunnel dual-choice tests. Bars indicate the number of complete flights to each volatile source (N = 40). Asterisks indicate significant differences within a choice test (χ^2 test, $**P < 0.01$; $***P < 0.001$). Undam. pl. = plants never exposed to caterpillar damage. Leaf-induced pl. = plants damaged on the three lower leaves by *S. exigua* larvae 48 hr prior to tests (damaged leaves removed). Recent Dam. = one leaf damaged by *H. zea* larvae 3 hr prior to tests. Recent damage was either added (+) or conducted (with) on the plants being tested.

df = 1). Females that made complete flights chose the leaf-induced plants 95% of the time when no recent damage was present, as opposed to 37.5% when a recently damaged leaf was added to the undamaged plant. *C. marginiventris* showed a clear preference for the undamaged plant + recent damage combination over the leaf-induced plant (Figure 3B).

Leaf-Induced Plant + Recent Damage Versus Undamaged Plant + Recent Damage. When a recently damaged leaf was added to both the leaf-induced and the undamaged plant, no clear preference for any combination was found in either parasitoid species (Figures 2C and 3C).

Leaf-Induced Plant with Recent Damage Versus Undamaged Plant with Recent Damage. When the recent damage was conducted on the leaf-induced plant itself, both parasitoids exhibited strong preference for this combination over the undamaged plant with recent damage combination (Figures 2D and 3D).

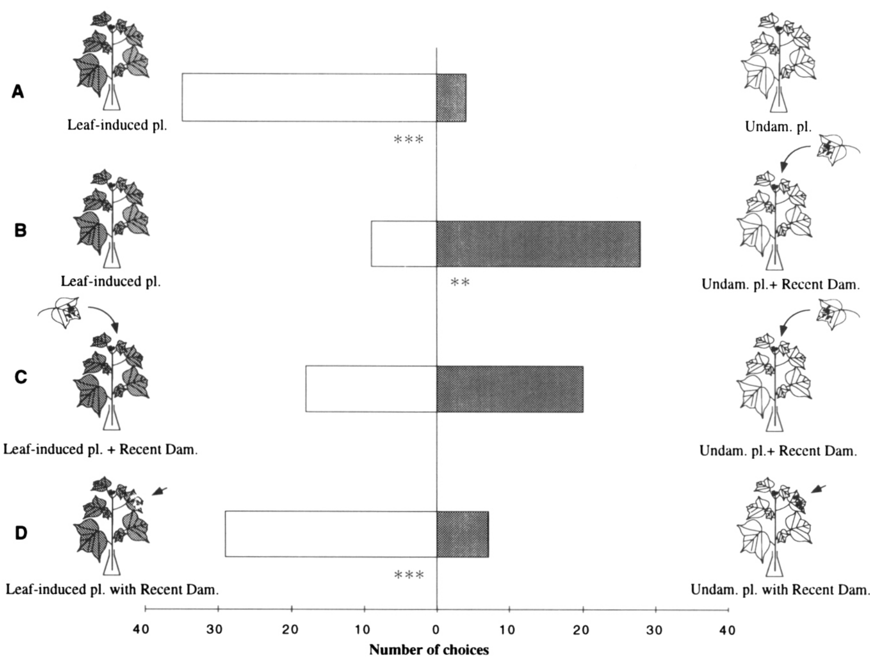

FIG. 3. Flight responses of *C. marginiventris* females in wind-tunnel dual-choice tests. Bars indicate the number of complete flights to each volatile source ($N = 40$). Asterisks indicate significant differences within a choice test (χ^2 test, $**P < 0.01$, $***P < 0.001$). Undam. pl. = plants never exposed to caterpillar damage. Leaf-induced pl. = plants damaged on the three lower leaves by *S. exigua* larvae 48 hr prior to tests (damaged leaves removed). Recent Dam. = one leaf damaged by *H. zea* larvae 3 hr prior to tests. Recent damage was either added (+) or conducted (with) on the plants being tested.

Leaf-Induced Plant Versus Undamaged Plant + Plant Frass. When frass obtained from cotton feeding larvae was added to undamaged plants, the results varied with parasitoid species. *M. croceipes* preferred the undamaged plant + plant frass combination (Figure 4B). Females with no previous oviposition experience exhibited similar preference (Figure 5C). *C. marginiventris*, however, displayed no particular preference (Figure 4D). When plant frass was added to undamaged plants, *C. marginiventris* preference for leaf-induced plants decreased (compare Figure 4C and D) ($\chi^2 = 6.2$, $P < 0.05$, $df = 1$). In that situation, the leaf-induced plant was chosen by only 58.3% of the females that made complete flights, against 86.8% when no frass was present.

Leaf-Induced Plant Versus Undamaged Plant + Artificial Frass (Naive Females). When frass obtained from artificial diet fed *H. zea* was added to undamaged plants, no significant difference was found in *M. croceipes* choices

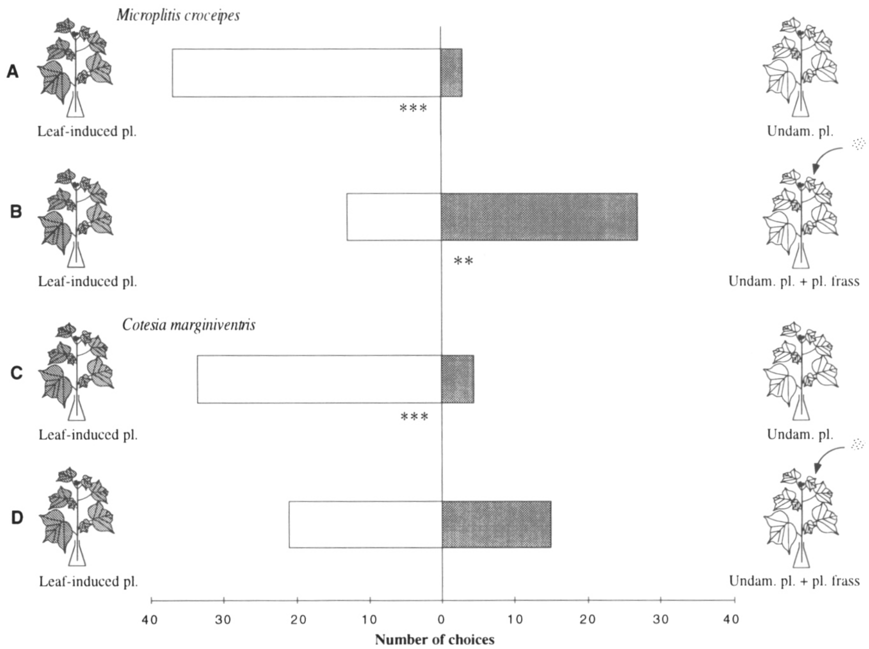

FIG. 4. Flight responses of *M. croceipes* (A, B) and *C. marginiventris* (C, D) females in wind-tunnel dual-choice tests. Bars indicate the number of complete flights to each volatile source ($N = 40$). Asterisks indicate significant differences within a choice test (χ^2 test, $^{**}P < 0.01$, $^{***}P < 0.001$). Undam. pl. = plants never exposed to caterpillar damage. Leaf-induced pl. = plants damaged on the three lower leaves by *S. exigua* larvae 48 hr prior to tests (damaged leaves removed). pl. frass = feces from *H. zea* larvae feeding on cotton plants for 24–36 hr.

between this combination and leaf-induced plants (Figure 5B). However, when artificial diet frass was added to undamaged plants, female preference decreased (compare Figure 5A and B) ($\chi^2 = 6.4$, $P < 0.05$, $df = 1$). Of the naive females that made complete flights, 87.5% chose the systemic plant when no frass was present compared to 60.0% when artificial diet frass was added to the undamaged plant.

Square-Induced Plant Versus Undamaged Plant. Plants induced by *H. zea* larvae feeding on squares had different effects on the two parasitoid species. *M. croceipes* preferred square-induced over undamaged plants (Figure 6B) but *C. marginiventris* showed no clear preference for either choice (Figure 6D). For the latter species, this was the treatment where the highest rate of incomplete flights (17.5%) was observed.

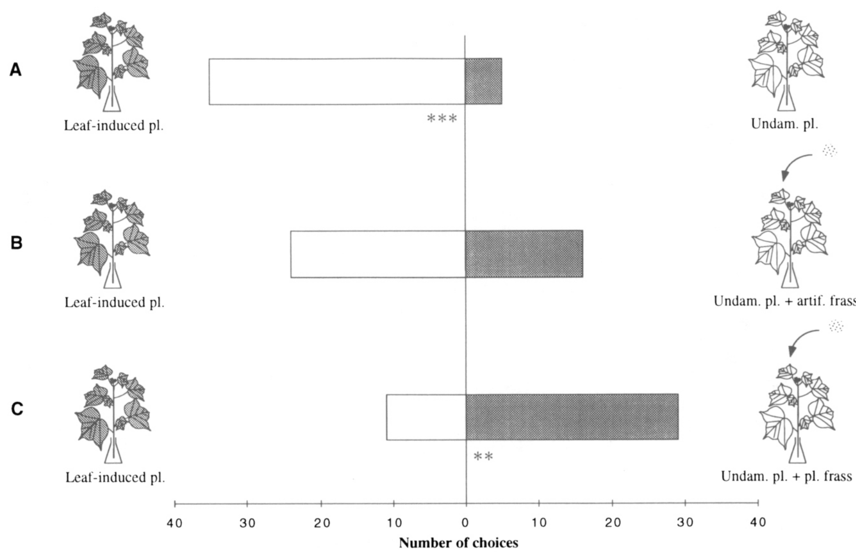

FIG. 5. Flight responses of naive *C. croceipes* females in wind-tunnel dual-choice tests. Bars indicate the number of complete flights to each volatile source ($N = 40$). Asterisks indicate significant differences within a choice test (χ^2 test, $^{**}P < 0.01$, $^{***}P < 0.001$). Undam. pl. = plants never exposed to caterpillar damage. Leaf-induced pl. = plants damaged on the three lower leaves by *S. exigua* larvae 48 hr prior to tests (damaged leaves removed). pl. frass = feces from *H. zea* larvae feeding on cotton plants for 24–36 hr. artif. frass = feces from *H. zea* larvae feeding on pinto bean diet for 24–36 hr.

DISCUSSION

Response to Leaf-Induced Plants. Despite the lack of previous plant experience, both parasitoid species were strongly attracted to volatiles released by undamaged terminals from cotton plants damaged by *S. exigua* larvae. When no other information was available, both *M. croceipes* and *C. marginiventris* strongly preferred leaf-induced plants over undamaged plants. Previous chemical analysis of cotton plants receiving similar *S. exigua* damage on the lower leaves demonstrated that such plants released herbivore-induced volatiles systemically (Röse et al., 1996). Our experimental design does not exclude the possibility of volatiles from damaged leaves being adsorbed on undamaged leaves and reemitted during wind-tunnel experiments. However, in all experiments, the induced plants and the undamaged plants were kept very close to each other. Any adsorption of volatiles from damaged sites would have occurred equally on both induced and undamaged plants. Thus, only systemically released vol-

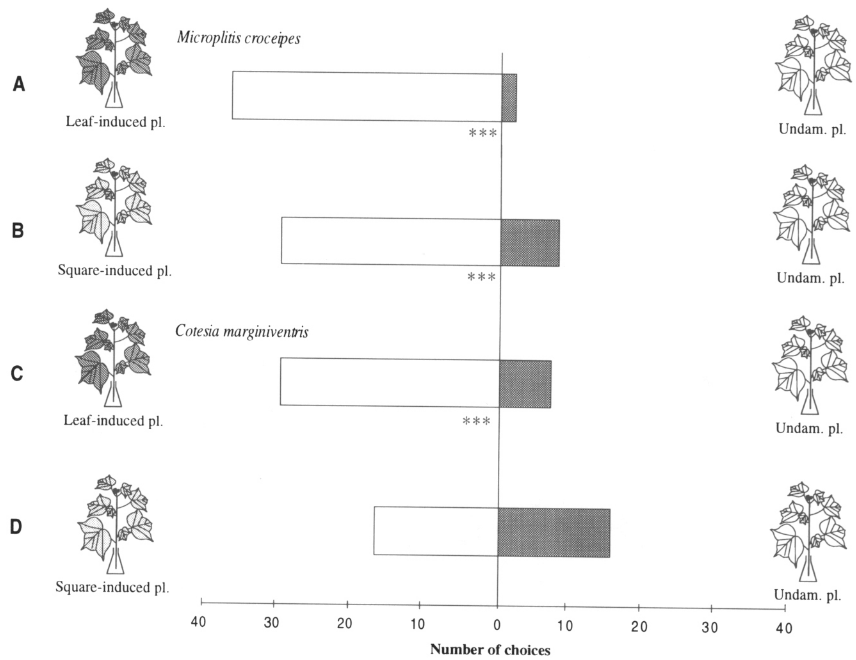

FIG. 6. Flight responses of *M. croceipes* (A, B) and *C. marginiventris* (C, D) females in wind-tunnel dual-choice tests. Bars indicate the number of complete flights to each volatile source ($N = 40$). Asterisks indicate significant differences within a choice test (χ^2 test, $***P < 0.001$). Undam. pl. = plants never exposed to caterpillar damage. Leaf-induced pl. = plants damaged on the three lower leaves by *S. exigua* larvae 48 hr prior to tests (damaged leaves removed). Square-induced pl. = cotton plants damaged on three or four squares by *H. zea* larvae 48 hr prior to the tests (damaged squares were removed).

atiles, as demonstrated by Röse et al. (1996), can reasonably account for the consistently stronger attraction of leaf-induced over undamaged plants.

Herbivore-induced systemically released volatiles are emitted from a large source and therefore, under natural conditions, may be detected over relatively large distances by parasitoids. These volatiles, which indicate herbivore damage, are likely to serve as long-range cues for the location of potential host patches. However, there is no evidence that volatiles emitted by undamaged portions of damaged plants carry information about the nature of the herbivore causing the damage. In our experiments, plants were damaged by *S. exigua* larvae, which are not suitable hosts for *M. croceipes*, and still these plants were very attractive. Turlings et al. (1993a) found similar results with corn seedlings induced with

S. exigua regurgitate. In these experiments, *M. croceipes* also showed strong attraction to non-host-induced corn seedlings. Furthermore, females were not able to distinguish between caterpillar- and grasshopper-induced seedlings. At the level of the actual damaged site, however, some plants emitted different volatiles when damaged by different herbivore species (Takabayashi et al., 1991; Agelopoulos and Keller, 1994b; Dicke, 1994). A number of parasitoids and predators are able to differentiate plants damaged by different species. The predatory mites *Phytoseiulus persimilis*, *Amblyseius potentillae*, and *Amblyseius finlandicus* can distinguish leaves infested by different spider-mite species (Sabelis and Van de Baan, 1983). The parasitoid *C. rubecula* showed no preference for cabbage plants damaged by hosts (*Pieris rapae*) or non-host caterpillars (*Plutella xylostella*) but preferred caterpillar- over snail- (*Helix aspera*) damaged plants (Agelopoulos and Keller, 1994a). However, host discriminative abilities at the damaged site level are no assurance of discriminative abilities at the systemic level, since the nature and the quantity of volatiles emitted locally and systemically may differ. Furthermore, learning can play an important role in parasitoids' abilities to discriminate between host and nonhost infested plants. Naive *C. glomerata* females did not discriminate between volatiles from plants infested by different caterpillar species (Geervliet et al., 1996). However, multiple experiences with a particular host plant complex led to clear preferences for this complex over others in wind-tunnel dual-choice tests (Geervliet, 1997). Similarly, the parasitoid *C. marginiventris* was able to distinguish between corn plants damaged by *S. exigua* or by *S. frugiperda* larvae after having an oviposition experience on either one of these host/plant complexes (Turlings et al., 1993b; Turlings et al., 1995). Therefore, further experimentation using *M. croceipes* and *C. marginiventris* with different learning experiences are needed to determine if they are able to distinguish between volatiles systemically released by plants damaged by different herbivore species.

Response to Other Plant- and Host-Related Signals. Once parasitoids enter potential host habitats, they cue on information more directly related to the presence of the host. Even if undamaged terminals from damaged plants are very attractive, parasitoids can still discriminate between signals emitted by these and signals indicating host presence more reliably. The nature of the signals used by the two parasitoid species studied here varied. Recent damage volatiles seemed to constitute the primary orientation cue for *C. marginiventris*. When a leaf damaged for less than 3 hr was added to an undamaged plant, this combination became more attractive to *C. marginiventris* than the systemic plant alone. On the other hand, when frass from *H. zea* larvae feeding on cotton leaves was added to an undamaged plant, *C. marginiventris* females exhibited no clear preference for either this combination or the systemic plant. Our results concur with previous findings by Turlings et al. (1991), where damaged corn seedlings were the primary source of volatiles attracting this parasitoid, whereas frass volatiles appeared to have only a minor role. Different results were found with *M. croceipes*, in which frass volatiles were the primary orientation cue. However, volatiles from recently damaged leaves also appeared to have some effect. When provided a choice between a leaf-induced plant and either an undamaged plant + a recently damaged leaf or an undamaged plant, *M. croceipes* females chose the undamaged plant + recent damage 12.5 times more often. In our experiments, we used leaves damaged for less than 3 hr. According to previous chemical analysis of cotton (McCall et al., 1994), volatiles emitted by recently damaged leaves are mainly composed of green-leaf volatiles and constitutive terpenes. These are emitted at the damaged site concomitant with caterpillar feeding (Loughrin et al., 1994) but are not emitted systemically (Röse et al., 1996). Herbivores such as lepidopterous larvae can move from previously damaged locations or fall prey to other predators. Such factors can reduce the predictability of discovering a host when cueing only on induced volatiles. However, because emission wanes with time, volatiles released immediately upon damage could constitute reliable indicators of proximate damage and therefore indicate actual host presence to foraging parasitoids.

Both *C. marginiventris* and *M. croceipes* were attracted by leaf-induced plants receiving new damage. At the plant patch level, these results indicate that induced plants receiving secondary damage may have some advantage over newly damaged neighbors by attracting more natural enemies and attracting them quicker. It is likely that leaves from plants already induced by previous herbivore damage release inducible volatiles faster when they receive new damage. Furthermore, recent chemical analysis with cotton (Röse et al., 1996) showed that artificially damaged systemic plants released larger amounts of inducible and constitutive volatiles than artificially damaged control plants. Previous studies with *M. croceipes* (McCall et al., 1993) and *C. marginiventris* (Turlings et al, 1993b) showed that both parasitoids preferred old damage to fresh damage. According to these authors, the absence of inducible volatiles in the recently damaged leaves could account for the preferences observed.

Response of Naive Females to Leaf-Induced Plants and to Host Frass. In our experiments, the number of *M. croceipes* failing to achieve complete flights remained low even when we used naive females. This confirms the strong effect of systemically released herbivore-induced volatiles on this species.

The active component in caterpillar frass appeared to have two origins. Although no preference was found between leaf-induced and undamaged plants + artificial frass, artificial diet frass volatiles appeared to have some attractive effect on *M. croceipes* females. When tested against a leaf-induced plant, an undamaged plant where artificial diet frass was added attracted this parasitoid 3.2 times more than an undamaged plant alone. Since artificial diet is not attractive to *M. croceipes* females, it can therefore be assumed that volatile compounds originating from the host itself and present in the frass are responsible for the attraction observed. However, adding plant frass resulted in a stronger attraction than adding artificial diet frass. Whether this resulted from an additive effect of plant and host products present in the frass or from an absence of modified plant-related products in the artificial diet frass remains unclear. The use of frass volatiles in the host location process of the specialist *M. croceipes* has already been reported (Jones et al., 1971; Eller et al., 1988; Lewis and Tumlinson, 1988). Previous experience with frass appeared to have a crucial influence on female response to these volatiles. Several studies (Lewis and Tumlinson, 1988; Lewis et al., 1991; Eller et al., 1992) showed that few naive *M. croceipes* would fly upwind to host feces even when the hosts were plant fed. However, our results demonstrate an innate attraction to host frass volatiles in *M. croceipes*. Because only choice experiments were conducted here and systemic volatiles (highly attractive even to naive females) were always present, it is conceivable that these volatiles were responsible for the flight initiation and initial orientation of naive females and that frass volatiles influenced females' choices at the shorter range. By being closer to situations encountered by foraging parasitoids in nature (frass volatiles would seldom be alone), our experiments allow a more realistic view of the use of multiple information. It should be noted that only humidified frass was used. This action increased the amount and modified the composition of volatiles detected by chemical analysis from *P. rapae* and *Pieris brassicae* frass (Agelopoulos et al., 1995).

In *M. croceipes*, frass volatiles represent a source of specific information allowing discrimination between host and nonhost species from a distance (Alborn et al., 1995). The presence of herbivore-specific cues for parasitoids in caterpillar frass has also been demonstrated for other species (Smith et al., 1994; Agelopoulos et al., 1995). For specialist parasitoids such as *M. croceipes*, the ability to detect volatiles specifically associated with their hosts could have a double advantage. Not only could it give indications at a distance of the identity of the species attacking a plant, but also, because of the extremely polyphagous nature of their hosts, it could help decrease the need to resort to multiple plant signals emanating from all the different species they feed on.

Response to Square-Induced Plants. M. croceipes can only parasitize *Heliothis/Helicoverpa* species that, on cotton, typically do not feed on leaves, but prefer squares, flowers, and bolls (Wilson and Gutierrez, 1980). As there is a strong correlation between this site being damaged and presence of a suitable host, volatiles specifically released by square-induced plants would be reliable cues for foraging *M. croceipes*. Our experiments show that the natural feeding behavior of *H. zea* larvae on cotton triggers the release of parasitoid-attracting volatiles from the undamaged parts of the plant. When cotton plants were induced by *H. zea* larvae feeding on squares, only *M. croceipes* showed a strong preference for these plants over undamaged plants. Since no chemical analysis has been done yet, the question remains whether the difference in volatile production between leaf-damaged plants and square-damaged plants is quantitative, qualitative, or both. At the damaged site level, Turlings et al. (1993b) found both qualitative and quantitative differences in the volatiles released by leaves, flowers, and bolls of cotton plants fed upon by *H. zea* larvae. So far, however, there is no evidence that such differences also exist at the systemic level. When *H. zea* larvae were contained on the squares with screened instead of closed cups, the responses of both parasitoids remained similar (Cortesero, unpublished data). Therefore, the difference between the response of *C. marginiventris* to leaf-induced and square-induced plants is not related to the method used to damage the plants. However, the origin of the lack of discriminative ability of *C. marginiventris* between *H. zea* square-induced plants and undamaged plants remains unclear. It may be related to differences in the species used to induce the plants as well as differences in the sites damaged. We hope to clarify this point in future experiments.

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